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(54) Title: REARRANGED SQUAMOUS CELL CARCINOMA ANTIGEN GENES II

(57) Abstract: The present invention relates to a SCCA1/SCCA2 fusion protein; plasmid containing the same; antibodies of said fusion protein; methods for detecting said protein; methods for diagnosing th epresence or absence of SCC by determining the presence of SCCA1/SCCA2 fusion protein.

REARRANGED SQUAMOUS CELL CARCINOMA ANTIGEN GENES II

DESCRIPTION

FIELD OF THE INVENTION

The present invention relates to a fusion protein transcript for the production of fusion protein, a fusion gene found in squamous cell carcinomas, detection of the rearrangement and monoclonal antibodies specific for SCCA1, SCCA1/A2, SCCA2/A1 and SCCA2.

BACKGROUND OF THE INVENTION

Squamous cell carcinoma antigen (SCCA) is a serological marker for squamous cell carcinomas (SCC) of the uterine cervix, lung, head and neck, vulva, and esophagus (1, 2). It was originally purified from the TA-4 complex from human cervical squamous cell carcinoma, with a molecular weight of 42-48 kDa (1, 3). The antigen consists of more than 10 proteins and iso-electric focusing of the antigen reveals two subfractions, an acidic (pI<6.25) and a neutral (pI≥6.25) isoform (4). The difference in molecular weight is probably due to modification (5).

Cloning of the cDNA of SCCA shows that it belongs to the family of serine protease inhibitors (serpins) (6). Further cloning of the genomic region on chromosome 18q21.3 reveals two tandemly arrayed genes (7). The more telomeric one, the original SCCA, was designated SCCA1, whereas the more centromeric one was designated SCCA2 (Figure 1A-C). They both contain eight exons and the putative intron-exon boundaries, splice sites, initiation codons, and terminal codons are identical. They are 98% identical at the nucleotide level (Figure 2) and 92% identical at the amino acid level (Figure 3). The deduced pI value shows that the neutral isoform is coded by SCCA1, and the acidic isoform by SCCA2. Alternatively spliced variant mRNA from both the genes have been found resulting in proteins 50 and 21 amino acids shorter (5).

In humans the serpins map to one of two chromosomal clusters. PI6, PI9 and ELNAH2 map to 6p25, whereas PI8, Bomapin, PAI2, SCCA1, SCCA2, Headpin and Maspin map to 18q21.3 (Figure 1B)(7-12). These clusters are supposed to have arisen via two independent interchromosomal duplications and several rounds of intrachromosomal duplications (9). The chromosome region 18q has often been reported as a region with high frequency of rearrangements (9, 13-16). The targets and functions of serpins are not fully understood. For

most, the primary functions are regulation of proteolytic events associated with coagulation, fibrinolysis, apoptosis and inflammation, but alternative functions such as hormone transport and blood pressure regulation have been reported (17-24).

Although SCCA1 and SCCA2 are nearly identical they differ in their reactive site loops (Figure 2 and 3). SCCA1 inhibits the papain-like cystein proteinases cathepsin S, K, and L (25, 26) while SCCA2 inhibits the chymotrypsin-like serine proteinases cathepsin G and mast cell chymase (27). Studies of the reactive site loop (RSL) of SCCA1 show that the RSL is essential for cystein proteinase inhibition (28). The variable portion of the RSL dictates the specificity of the target proteinases shown by RSL swap mutants of SCCA1 and SCCA2 and single mutants (28, 29). It is likely that serpins utilize a common RSL-dependent mechanism to inhibit both serine and cystein proteinases.

The biological role of SCCA1 and SCCA2 are not fully understood. They are considered to be inhibitory serpins. Data suggest that SCCA1 is involved in apoptosis and expression makes cancer cells resistant to several killing mechanisms by inhibition of apoptosis (30). The role of SCCA2 expression in cancer cells is still unclear. In normal tissue SCCA antigen may have some specific role during epidermal maturation (5).

Recent studies using discriminatory monoclonal antibodies and polymerase chain reaction (PCR) have shown that both SCCA1 and SCCA2 are expressed in the suprabasal layers of the stratified squamous epithelium of the tongue, tonsil, esophagus, uterine cervix and vagina, Hassall's corpuscles of the thymus, some area of the skin and in the stratified columnar epithelium of the conducting airways (31). In squamous cell carcinomas of the lung and head and neck, SCCA1 and SCCA2 were co-expressed in moderately and well-differentiated tumors. In contrast to previous studies using nondiscriminatory antibodies, these data show that there were no differential expression between SCCA1 and SCCA2 in normal and malignant tissue. Previous results have shown that SCCA2 was only detected at the peripheral parts of the tumor (32). This discrepancy may be due to differences between immunohistochemical techniques and antibody specificities (31). It has been reported that false positive results may often be caused by contamination with saliva or sweat during assay procedure (1). Cataltepe et al. suggest that the SCCAs in saliva are derived from the squamous epithelial cells lining mucosal surfaces of the upper digestive tract (31).

Normally, SCCA1 and SCCA2 are detected in the cytoplasm of squamous epithelial cells (31), but not in the circulation (33). The antigen, which appears in the serum of patients with SCC, may be a function of SCCA-over-production by tumor cells and their normal turn over (34). It has been reported that the SCCA detected in serum by using antibody radioimmuno-assay or RT-PCR is mainly SCCA2 (1, 35, 36) but other studies using PCR indicate that both antigens can be amplified and detected in patient samples (37).

Serum concentrations present in patients with SCC are correlated to the clinical stage and to the degree of histological differentiation of the tumor (1). For cervical cancer several studies show a correlation between the pretreatment values and the clinical outcome (1, 38-43). Studies also show a correlation between high SCCA levels and tumor volume. Recurrence or progressive disease could be detected several months before clinical evidence (39). Similar results are seen for squamous cell carcinomas of the lung, vulva, head and neck and esophagus (1, 2, 44, and 45). In all these studies, they have measured the total SCCA level. Recently a new sELISA was developed using discriminating antibodies for SCCA1 and SCCA2 (33).

SUMMARY OF THE INVENTION

The present invention relates to fusion protein transcipts, mRNA, of different genes to provide a fusion protein, and in particular it provides the detection of a fusion gene consisting of parts of SCCA1 and SCCA2. Such fusion genes have now been found in SCC cell-lines of different origin (cervix, lung and pharynx). The invention also provides methods for establishment of specific immunological reagents for determination/detection of the fusion proteins.

One fusion protein is defined by the following amino acid sequence

MNSLSEANTK FMFDLFQQFR KSKENNIFYS PISITSALGM VLLGAKDNTA QQIKKVLHFD QVTENTTGKA ATYHVDRSGN VHHQFQKLLTE FNKSTDAYE LKIANKLFGE KTYLFLQEYL DAIKKFYQTS VESVDFANAP EESRKKINSW VESQTNEKIK NLIPEGNIGS NTTLVLVNAI YFKGQWEKKF NKEDTKEEKF WPNKNTYKSI QMMRQYTSFH FASLEDVQAK VLEIPYKGKD LSMIVLLPNE IDGLQKLEEK LTAEKLMEWT SLQNMRETCV DLHLPRFKME ESYDLKDTLR TMGMVNIFNG DADLSGMTWS HGLSVSKVLH KAFVEVTEEG VEAAAATAVV VVELSSPSTN EEFCCNHPFL FFIRQNKTNS ILFYGRFSSP

based upon the DNA sequence

ATGAATTCAC TCAGTGAAGC CAACACCAAG TTCATGTTCG ACCTGTTCCA
ACAGTTCAGA AAATCAAAAG AGAACAACAT CTTCTATTCC CCTATCAGCA
TCACATCAGC ATTAGGGATG GTCCTCTTAG GAGCCAAAGA CAACACTGCA
CAACAGATTA AGAAGGTTCT TCACTTTGAT CAAGTCACAG AGAACACCAC
AGGAAAAGCT GCAACATATC ATGTTGATAG GTCAGGAAAT GTTCATCACC
AGTTTCAAAA GCTTCTGACT GAATTCAACA AATTCCACTGA TGCATATGAG
CTGAAGATCG CCAACAAGCT CTTCGGAGAA AAAACGTATC TATTTTTACA
GGAATATTTA GATGCCATCA AGAAATTTTA CCAGACCAGT GTGGAATCTG
TTGATTTTGC AAATGCTCCA GAAGAAAGTC GAAAGAAGAT TAACTCCTGG
GTGGAAAGTC AAACGAATGA AAAAATTAAA AACCTAATTC CTGAAGGTAA
TATTGGCAGC AATACCACAT TGGTTCTTGT GAACGCAATC TATTTCAAAG
GGCAGTGGGA GAAGAAATTT AATAAAGAAG ATACTAAAGA GGAAAAATTT
TGGCCAAACA AGAATACATA CAAGTCCATA CAGATGATGA GGCAATACAC
ATCTTTTCAT TTTGCCTCGC TGGAGGATGT ACAGGCCAAG GTCCTGGAAA
TACCATACAA AGGCAAAGAT CTAAGCATGA TTGTGTTGCT GCCAAATGAA
ATCGATGGTC TCCAGAAGCT TGAAGAGAAA CTCACTGCTG AGAAATTGAT
GGAATGGACA AGTTTGCAGA ATATGAGAGA GACATGTGTC GATTTACACT
TACCTCGGTT CAAAATGGAA GAGAGCTATG ACCTCAAGGA CACGTTGAGA
ACCATGGGAA TGGTGAATAT CTTCAATGGG GATGCAGACC TCTCAGGCAT
GACCTGGAGC CACGGTCTCT CAGTATCTAA AGTCCTACAC AAGGCCTTTG
TGGAGGTCAC TGAGGAGGGA GTGGAAGCTG CAGCTGCCAC CGCTGTAGTA
GTAGTCGAAT TATCATCTCC TTCAACTAAT GAAGAGTTCT GTTGTAATCA
CCCTTTCCTA TTCTTCATAA GGCAAAATAA GACCAACAGC ATCCTCTTCT
ATGGCAGATT CTCATCCCCA TAGATGCAAT TAGTGTGTCA CT

DESCRIPTION OF SPECIFIC EMBODIMENTS

One fusion gene (Figure 4) was found by sequencing cDNA from SCC cell lines.

Cell line	Origin	SCCA1	SCCA2
CaSki	Cervix	normal	A1/A2
C4I	Cervix	normal	normal

A549	Lung	N.A.	A1/A2
CaLu3	Lung	normal	normal
SkMES	Lung	normal	normal
RPMI2650	Pharynx	N.A.	A1/A2

According to the sequence swift from SCCA1 to SCCA2, the DNA breakpoint would be in intron 7 (Figure 2). The gene should consequently be controlled via the promoter region of SCCA1 but producing a protein with SCCA2-specificity.

Further investigations have shown that a fusion protein transcript occurs at different sites when it comes to SCCA1/A2 or SCCA2/A1, i.e., the different genes of these antigens provide cross-over transcripts having the promoter region of one gene and the reactive site loop of another gene.

This leads to the basic invention related to a fusion protein transcript of two different genes having a nucleotide homology of at least 80% in a certain region, the region of cross-over reaction.

The fusion genes are cloned and kept as plasmid-constructs as well as transformed into different E. coli strains.

A plasmid, pGEX6P-3 SCCA1/A2, containing the fusion gene has been deposited with European Collection of Cell Cultures on the 14th of March, 2001, under deposition number ECACC 01031315.

Fusion protein has been produced and complex binding studies show substrate binding of the fusion gene to Cathepsin G but not to Cathepsin L (Figure 9).

The fusion gene can be detected by Southern blot analysis of tumor DNA (Figure 8). The fusion gene can also be detected by PCR analysis as well as by cDNA cloning and sequencing.

EXAMPLE 1

Cloning of SCCA

1. 1. PCR amplification

mRNA from the cell-lines Caski (cervix), C4-I (cervix), A549 (lung), CaLu3 (lung), SkMes (lung), and RPMI2650 (pharynx) was prepared using QuickPrep Micro mRNA Purification kit (Pharmacia) and cDNA was prepared using First-Strand cDNA Synthesis kit (Pharmacia). A 1218bp DNA fragment covering the coding sequence of SCCA was amplified by PCR in a 100 µl reaction containing 10 mM Tris-HCl pH 8.85, 25 mM KCl, 5 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$ (Boehringer), 0.2mM dNTP (Pharmacia), 10 µM SCCA 1-7F (DNA sequences for all primers are shown in Table 1), 10 µM SCCA 391-397B, 2 µl cDNA and 2.5 U Pwo-polymerase (Boehringer). After denaturing samples for 5 min at 96°C a total of 30 cycles were performed, each consisting of denaturation for 15 sec at 96°C, annealing for 15 sec at 60°C, and extension for 30 sec at 72°C. The PCR reaction was completed by a final extension for 10 min at 72°C.

TABLE 1. PCR-primers

Primer name	Sequence
1. SCCA 1-7F	5'-CGGGATCCATGAATTCACTCAGTGAAGCC-3'
2. SCCA 391-397B	5'-GAGCTCGAGTCTCATCAGTGACAGACTAATTGCATCTA-3'
3. SCCA 266-273F	5'-TGGAATGGACAAGTTTGCAG-3'
4. SCCA1 323-329B	5'-GTAGGACTCCAGATAGCAC-3'
5. SCCA2 319-324F	5'-TGGAGCCACGGTCTCTCAG-3'
6. SCCA2 357-363B	5'-ATTAGTTGAAGGAGATGATAATTC-3'
7. SCCA1 ex7	5'-AATACATACAAGTCCA-3'
8. SCCA2 ex8	5'-GGACTTTAGATACTGA-3'

1. 2. Detection of SCCA1 and SCCA2

Presence of SCCA1 in PCR products were detected by cleavage with restriction enzyme SacII, resulting in two fragments, 245 and 973 bp, respectively, or by SCCA1-specific PCR using the primers SCCA1-7F and SCCA1 323-329B in a standard PCR reaction (75 mM Tris-HCl pH 8.8, 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20, 2 mM $MgCl_2$, 0.2 mM dNTP, 10 µM of each primer, template, and 0.025 U/µl reaction Taq Polymerase; after denaturing samples for 5 min at 96°C a total of 30 cycles were performed, each consisting of denaturation for 15 sec at 96°C, annealing for 15 sec at optimal annealing temperature, and extension for 30 sec at 72°C. The PCR reaction was completed by a final extension for 10 min at 72°C.), Ta=50°C, resulting in a 997 bp fragment. Presence of SCCA2 were detected by standard PCR using

SCCA 1-7F and an SCCA2-specific primer, SCCA2 357-363B, Ta=60°C, giving a 1090 bp fragment.

1. 3. Cloning

PCR-products were cloned using PCR-Script Amp cloning kit (Stratagene). Colony screening were performed by PCR as described in 1.2 above.. Plasmid-DNA was prepared from selected clones containing SCCA1 or SCCA2 using Wizard Plus Minipreps DNA Purification System (Promega).

1. 4. DNA sequencing

Clones were sequenced using ABI Prism BigDye Terminator Cycle Sequencing (PE Biosystems). Samples were run on an ABI Prism 310.

1. 5. Recloning

Selected clones were recloned into the expression vector pGEX-6P-3 (Pharmacia). Fragments were excised from the PCR-Script Amp vector using BamHI and XhoI and ligated into the expression vector in a 10 μl reaction containing 1xOPA, 1 mM ATP, 50 ng cleaved vector, SCCA insert corresponding to a moles-of-ends vector: insert ratio of 1:5–1:8, and 7.5-10 U T4DNAligase (all from Pharmacia). Reaction tubes were incubated at 10°C overnight and inactivated for 10 min at 65°C. 2-4 μl of the reaction was transformed into E.coli JM109 (46). Plasmid-DNA from selected clones were then transformed into E.coli BL21 for protein expression.

1. 6. Maintenance of cloned gene

Plasmid-DNA (pGEX-6P-3 containing the SCCA1/A2 fusion gene) in a 10 mM Tris-HCL pH 8.0 buffer solution is stored in –80°C. For resuming protein expression, plasmid-DNA are transformed into competent E.Coli BL21 according to Sambrook et al. (p 1.82-1.84 in ref. 45). For preparation of more plasmid-DNA, transformation into E. Coli JM109 is preferred.

EXAMPLE 2

Protein expression and purification

2. 1. Protein Expression

Expression conditions were determined by small-scale preparations. For large scale expression 500 ml cultures of 2xYT and 100 μg/ml ampicillin were inoculated with 5 ml

over-night culture and grown at 37°C. Protein expression was induced at OD_{600}=0.5-1.3 by adding IPTG to a final concentration of 0.1 mM. Cultures producing SCC1 were grown for 4-16 h, SCCA1/A2 for 16-18 h. Cultures producing the SCCA2 protein were induced at OD_{600}= 1.2-1.4 and were grown for 2-3 h.

2. 2. Protein Purification

Cells were harvested by centrifugation for 10 min at 2000 g, washed with 50 ml TE pH 8.0, and dissolved in 3 ml TE/g bacterial pellet. Lysozyme was added to a final concentration of 800 μg/g pellet and the mixtures were incubated on ice for 30-60 min and then frozen over night at –70°C. Magnesium chloride and DNase were added to a final concentration of 12 mM and 20 μg/g pellet, respectively. After incubation on ice for 30 min, samples were centrifuged for 30 min at 40000 g. To each supernatant 0.5 ml of 50% Glutathione Sepharose (Pharmacia) was added and incubated for 30 min-2 h at room temperature with gentle agitation. The slurry was washed 5-7 times using 1xPBS. GST-SCCA fusion protein was eluted using 0.5-1 ml Reduced Glutathione (Pharmacia) and incubated for 30-60 min at room temperature or over-night at 4°C, all with gentle agitation. SCCA protein was eluted by cleavage in between GST and SCCA. 0.48 ml cleavage buffer (50 mM Tris-HCl pH 7.0, 150 mM NaCl, 1 mM EDTA, 1 mM DTT) and 20μl PreScission protease were added and samples were incubated at 4°C with gentle agitation for 4 h or over-night. Proteins were analyzed on SDS-PAGE by Phast-system (Pharmacia).

2 3. Complex binding

Complex binding of SCCA to substrates was performed by mixing 2 μg of SCCA-protein with 0.5 μg of Cathepsin G (Biodesign Int.) or 0.5 μg of 0.9 μg Cathepsin L (Calbiochem) in 1xPBS buffer in a total volume of 4.5 μl. Samples were incubated at 37°C for 30 minutes. To each sample, 0.5 μl of 10xComplex-buffer (20% SDS, 140 mM Mercaptoethanol, bromophenolblue) was added. Samples were incubated for 3 minutes at 95°C and analyzed on a 12.5% SDS-PAGE-gel. The SCCA1/A2 fusion protein forms a complex with Cathepsin G but not with Cathepsin L showing that the fusion protein is functional and has the substrate specificity of SCCA2 (Figure 8).

EXAMPLE 3

DNA analysis

3. 1. Southern Blot Analysis

Approximately 10 μg of DNA prepared from SCC cell-lines as well as from blood samples from normal healthy volunteers, were digested with restriction endonucleases PstI or BamHI. Digested DNA were separated on 0.8 % agarose and transferred to membranes (Hybond N+, Pharmacia). Filters were prehybridized for 1 h and hybridized over night at 60°C in 20 ml of a solution containing 5xSSC, 0.1% SDS, 5% Dextrane sulfate, Liquid block (Pharmacia) diluted 1:20 and salmon sperm DNA 100μg/ml. Probe concentration during hybridization was 10 ng/ml. After hybridization filters were stringency washed for 15 min in 1xSSC/0.1%SDS and for 15 min in 0.2xSSC/1%SDS, both at 60°C. Probe hybridization was detected using Gene Images CDP-Star detection module (Pharmacia) with minor modifications. Filters were blocked for 1 hour at room temperature in a solution containing liquid block diluted 1:7.5. Then they were incubated in buffer A (0.1M Tris, 0.3M NaCl, pH 9.5) / 0.5% BSA for 15 min before adding the anti-fluorescein HRP conjugate diluted 1:6800 and then incubated for another 45 min. Filters were washed for 3x10 min in buffer A/0.3% Tween 20 before adding detection reagent. Filters were incubated for 2 min, washed briefly in 2xSCC and wrapped in plastic film. Hyperfilm MP was exposed for 35 min.

3. 2. Hybridization probes

Probes were generated and labeled by PCR in a reaction containing 60 μm each of dATP, dCTP, and dGTP, 24 μM dTTP, 40 μM Fluorescein-11-dUTP, 2 mM $MgCl_2$, 3μM forward primer, 3 μM backward primer, 15 ng DNA template (SCCA2-containing plasmid), 1 U Taq polymerase and 1xPCR buffer (Advanced Biotechnologies). Probe I: A 393 bp fragment of exon 8 (nucleotide 802-1194), primers SCCA 266-273F and SCCA 391-397B, Ta=50°C; Probe II: A 126 bp fragment of exon 8 (nucleotide 957-1082), primers SCCA2 319-324F and SCCA2 357-363B, Ta=50°C; probe III: A 1194 bp fragment covering the coding sequence and 22 nucleotides in the 3'-end of the gene, primers SCCA 1-7F and SCCA 391-397B, Ta=60°C.

Southern blot of PstI digested DNA hybridized with probe I show a different band pattern of DNA from a SCC-cell line compared to that of normal control DNA (Figure 9). DNA digested with BamHI also shows aberrant bands compared to normal control DNA.

3. 3. PCR analysis

DNA isolated by routine procedures from samles analysed by PCR using primers 7 and 8 (see Table 1) in a standard PCR-reaction show only product in samples containing the fusion gene.

EXAMPLE 4

Hybridomas and monoclonal antibodies

4. 1. Establishment of hybridomas and production of monoclonal antibodies reactive with SCCA1/A2, SCCA2 and SCCA1

Polyclonal antisera reactive with SCC antigen was obtained by subcutaneous immuniza-tion of Rabbits with recombinant SCC antigen and collection of immune sera according to standard procedures. The titer of the polyclonal antisera was tested by determination of the reactivity of the antisera with biotinylated SCCA1/A2 and SCCA1 immobilized in streptavidin plates (Labsystems Oy, Helsinki, Finland), Figure 6. The recombinant SCCA1/A2 and SCCA1 were biotinylated with Biotin-N-succinimide caproate ester according to standard procedures.

Monoclonal antibodies reactive with SCCA1/A2 and SCCA2 were established by immunization of Balb/c mice intra peritoneally with 10 - 50 μg of recombinant SCCA1/A2 in Ribi adjuvant. After the immunization and 2 - 4 booster doses during 60 - 90 days spleen cells from the immunized mice were fused with P3 x 63Ag 8 myeloma cells as described (47).

Hybridomas producing antibodies reacting with SCCA1/A2 were selected by ELISA screening of hybridoma supernatants in microtiter wells coated with affinity purified polyclonal antiserum against mouse IgG + M, (Jackson Immuno Res Lab, US). The wells were then incubated with SCCA1/A2 antigen, and after washing the bound antigen was detected by incubation with polyclonal Rabbit Anti SCC and HRP labeled Swine Anti Rabbit Ig (Dako AS, Copenhagen, Denmark).

4. 2. Reactivity of selected hybridomas with SCC antigens

The reactivity of the established hybridomas was tested in an ELISA similar to the ELISA screening procedure. Briefly the monoclonal antibodies produced by the hybridomas were immobilized in microtiter plates coated with polyclonal antiserum against mouse IgG+M (Jackson Immuno Res Lab, US). The wells were then incubated with 50 μL of the different recombinant SCC antigens in PBS 1% BSA for 1 h, after washing the plates were incubated with 100 μL Rabbit antiSCC diluted 1/5000 in PBS-1%BSA and incubated for additional 1h.

The bound Rabbit Anti-SCC was then detected by incubation with HRP - Swine anti Rabbit Ig and visualized with OPD substrate and determination of OD at 450 nm.

In figure 7 the reactivity of selected hybridomas are shown. The SCC106, SCC114, SCC115 reacted only with SCCA1/A2, which indicate that they are specific for the SCCA1/A2 fusion protein. The SCC100, SCC103 and SCC109 reacted with SCCA2 and SCCA1/A2 but not with SCCA1 indicating that they are specific for SCCA2. The SCC110, SCC111 and SCC124 reacted with SCCA1 and SCCA1/A2 but not with SCCA2 suggesting that they are specific for SCCA1.

The SCC107, SCC119 and SCC128 reacted with all SCC antigens suggesting that they recognize a common epitope in SVVA1 and SCCA2.

Twice limiting dilution cloned clones producing antibodies reacting with SCCA1/A2, but negative for SCCA1 were produced.

Monoclonal antibodies were produced by in vitro cultivation of the hybridoma clones by inoculation of 10^4 cells/mL in DMEM, 5 % Fetal Calf Serum in roller bottles and allowed to grow for 10 - 14 days. The monoclonal antibodies were then purified from the culture medium by Protein A (Bioprocessing Ltd, Durham, UK) affinity chromatography according to the manufacturers recommendation.

EXAMPLE 5

Using the established monoclonal antibodies and recombinant proteins it was possible to develop immunoassays for specific determination of SCCA1/A2 fusion protein and assays specific for SCCA2 and SCCA1 respectiviely.

5. 1 Immunoassays for determination of SCCA1/A2 fusion protein

Assays specific for SCCA1/A" fusion protein but essentially negative for SCCA1 and SCCA2 were designed by using antibodies among SCC106, SCCC114 or SCC115 in combination with antibodies among SCC107, SCC119 or SCC128, see figure .

In the preferred configuration antibody SCC107 was used as catching antibody and SCC106 as detecting antibody.

SCC107 MAb was biotinylated with BiotinNHRS caproate ester, Sigma Chemical Co, US, using standard procedures, and used as catching antibody. SCC106 MAb were conjugated with HRP according to a modification of the Nakone procedure.

The biotinylated SCC107 MAb and HRP conjugated SCC106 MAb were used in two-site EIA according to the following protocol.

Assay procedure

1. Add 50 µL of SCCA recombinant antigen (0 - 100 µg/L in PBS, 60 g/L BSA, pH 7.2) + 100 µL of Biotin SCC107 MAb, 2 µg/mL, in Assay Buffer in Streptavidin coated microtiter plates, Labsystems Oy, Helsinki, Finland.
2. Incubate for 1 h ± 10 min with shaking
3. Wash 3 times with 5 mM Tris buffer, 0.05 % Tween 40, pH 7.75.
4. Add 100 µL HRP SCC106 MAb, 2 µg/mL, in Assay Buffer.
5. Incubate for 1 h ± 10 min with shaking.
6. Wash 6 times with 5 mM Tris buffer, 0.05 % Tween 40, pH 7.75.
7. Add 100 µL TMB, ELISA Technology, US.
8. Incubate 30 min± 5 min
9. Determine OD 620 nm in ELISA reader.

Dose-response curves for SCCA1, SCCA2 and SCCA1/A2 antigens revealed that the assay was specific for the SCCA1/A2 recombinant antigen with < 5 % cross reactivity with SCCA1 or SCCA2.

5. 2 Assays for specific determination of SCCA2

Assays specific for SCCA2 without significant reactivity with SCCA1/A2 and SCCA1 were designed by using antibodies among SCCC100, SCC103 or SCC109 in combination with antibodies among SCC107, SCC119 or SCC128. In the preferred configuration SCC107 MAb was used as catching antibody and the SCC103 was used as detecting antibody.

SCC107 MAb was biotinylated with BiotinNHRS caproate ester (Sigma Chemical Co, US) using standard procedures, and used as catching antibody. SCC103 MAb was conjugated with HRP, Type V (Sigma Chemical Co, US), according to a modification of the Nakone procedure.

The biotinylated SCC107 MAb and HRP conjugated SCC103 MAb were used in two-site EIA according to the following protocol.

Assay procedure:

1. Add 50 µL of SCC recombinant antigen (0 - 100 µg/L in PBS, 60 g/L BSA, pH 7.2) + 100 µL
2. Incubate for 1 h ± 10 min with shaking
3. Wash 3 times with 5 mM Tris buffer, 0.05 % Tween 40, pH 7.75.
4. Add 100 µL HRP SCC103 MAb 2 µg/mL, in Assay Buffer.
5. Incubate for 1 h ± 10 min with shaking.

6. Wash 6 times with 5 mM Tris buffer, 0.05 % Tween 40, pH 7.75.
7. Add 100 µL TMB, ELISA Technology, US
8. Incubate 30 min± 5 min
9. Determine OD 620 nm in ELISA reader.

Based on the dose-response curves for SCCA2, SCCA1 and SCCA1/A2 fusion protein it was concluded that the assay according to example 5.2 was specific for SCCA2 with a cross-reactivity of < 5 % for SCCA1 and SCCA1/A2.

5. 3. Assays for specific determination of SCCA1

Assays specific for SCCA1 without significant reactivity with SCCA2 and SCCA1/A2 were designed by using antibodies among SCC110, SCC111 or SCC124 in combination with antibodies of among SCC107, SCC119 or SCC128. In the preferred configurations SCC107MAb was used as catching antibody and SCC124 MAb was used as detecting antibody.

SCC107 MAb was biotinylated with BiotinNHRS caproate ester (Sigma Chemical Co, US) using standard procedures, and used as catching antibody. SCC124 MAb was conjugated with HRP, Type V, (Sigma Chemcal Co., US) according to a modification of the Nakone procedure.

The biotinylated SCC107 MAb and HRP conjugated SCC124 MAb were used in two-site EIA according to the following protocol.

Assay procedure:

Add 50 µL of SCC antigen (0 - 100 µg/L in PBS, 60 g/L BSA, pH 7.2)

+ 100 µL of Biotin SCC107 MAb, 2 µg/mL, in Assay Buffer in Streptavidin coated microtiter plates (Labsystems Oy, Helsinki, Finland).

2. Incubate for 1 h ± 10 min with shaking
3. Wash 3 times with 5 mM Tris buffer, 0.05 % Tween 40, pH 7.75.
4. Add 100 µL HRP SCC124 MAb, 2 µg/mL, in Assay Buffer.
5. Incubate for 1 h ± 10 min with shaking.
6. Wash 6 times with 5 mM Tris buffer, 0.05 % Tween 40, pH 7.75.
7. Add 100 µL TMB, (ELISA Technology, US).
8. Incubate 30 min± 5 min
9. Determine OD 620 nm in ELISA reader.

Based on the antibodies according to 5.3 immunoassays specific for SCCA1 with < 10 % cross-reactivity for SCCA2 or SCCA1/A2 antigen may be designed.

FIGURE LEGENDS

1. Chromosome 18 rearrangement

2. Alignment of the coding DNA regions, exon 2-8 of SCCA1 and SCCA2. Intron positions indicated -Ix-. Differences between the genes are indicated in grey. Italic letters show the regions coding for reactive site loops. Arrows show primer (Table 1) positions.

3. Alignment of protein sequences of SCCA1 and SCCA2. Intron positions are indicated with dotted lines. Differences between the proteins are indicated in grey tone. Boxes show the reactive site loops.

4. Nucleotide coding DNA region, exon 2-8 of the rearranged SCCA1/SCCA2. Sequences derived from SCCA1 are shown in normal style while sequences derived from SCCA2 are shown in bold. Intron positions are indicated in -Ix-. Differences between the genes are indicated in grey. Italic letters show the region coding for reactive site loop.

5. Protein sequence of the SCCA1/SCCA2 fusion protein. Amino acids derived from SCCA1 are shown in normal letters. Amino acids derived from SCCA2 are shown in bold letters. Intron positions are indicated with dotted lines. Differences between the proteins are indicated in grey. The reactive site loop is marked with a box.

6. Titer of PABan to SCC antigen.

7. Reactivity of established hybridomas with different SCC antigens.

8. Complex-binding analysis of SCCA1/A2 fusion protein. Lane A: SCCA1/A2, Lane B: SCCA1/A2 incubated with Cathepsin G, Lane C: SCCA1/A2 incubated with Cathepsin L. The complex of SCCA1/A2 and Cathepsin G is indicated by an arrow. Molecular weight marker is indicated.

9. Southern blot analysis of genomic DNA digested with PstI and hybridized with probe I. Lane A: RPMI2650 containing the SCCA1/SCCA2 fusion gene, Lane B:Normal DNA. Aberrant bands are indicated with arrows. Molecular weight marker is indicated.

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CLAIMS

1. Fusion transcript consisting of a homologue cross-over between two different genes with more than 80% sequence homology in certain regions, in particular regions of cross-over.

2. Fusion transcript according to claim 1, wherein the two genes are the genes of SCCA1 and SCCA2.

3. Full length fusion transcript protein between SCCA1 and SCCA2 having switched reactive site loops compared to basic promoter.

4. Substantially full length fusion transcript protein between SCCA1 and SCCA2 having switched reactive site loops compared to basic promoter.

5. A fusion protein according to claim 4 coded by one or more of exons 2-7 of SCCA1 gene fused to exon 8 of SCCA2 gene.

6. A fusion protein according to claim 1 coded by exon 2 – 7 of SCCA1 gene fused to exon 8 of SCCA2 gene.

7. A fusion protein according to claim 4 coded by one or more of exons 2-7 of SCCA2 gene fused to exon 8 of SCCA1 gene.

8. A fusion protein according to claim 1 coded by exon 2 – 7 of SCCA2 gene fused to exon 8 of SCCA1 gene.

9. A fusion protein according to claim 5, wherein the protein sequence is

MNSLSEANTK FMFDLFQQFR KSKENNIFYS PISITSALGM VLLGAKDNTA QQIKKVLHFD QVTENTTGKA ATYHVDRSGN VHHQFQKLLTE FNKSTDAYE LKIANKLFGE KTYLFLQEYL DAIKKFYQTS VESVDFANAP EESRKKINSW VESQTNEKIK NLIPEGNIGS NTTLVLVNAI YFKGQWEKKF NKEDTKEEKF WPNKNTYKSI QMMRQYTSFH FASLEDVQAK VLEIPYKGKD LSMIVLLPNE IDGLQKLEEK LTAEKLMEWT SLQNMRETCV DLHLPRFKME ESYDLKDTLR TMGMVNIFNG DADLSGMTWS HGLSVSKVLH KAFVEVTEEG VEAAAATAVV VVELSSPSTN EEFCCNHPFL FFIRQNKTNS ILFYGRFSSP

10. A fusion protein sequence according to claim 3, wherein the sequence is TAVV VVELSSPST

11. A DNA sequence coding for a fusion SCCA1/SCCA2 protein.

12. A DNA sequence comprising the nucleotide sequence of exon 2-7 of SCCA1 fused to the nucleotide sequence of exon 8 of SCCA2.

13. A DNA sequence according to claim 12, wherein the nucleotide sequence is

ATGAATTCAC TCAGTGAAGC CAACACCAAG TTCATGTTCG ACCTGTTCCA
ACAGTTCAGA AAATCAAAAG AGAACAACAT CTTCTATTCC CCTATCAGCA
TCACATCAGC ATTAGGGATG GTCCTCTTAG GAGCCAAAGA CAACACTGCA
CAACAGATTA AGAAGGTTCT TCACTTTGAT CAAGTCACAG AGAACACCAC
AGGAAAAGCT GCAACATATC ATGTTGATAG GTCAGGAAAT GTTCATCACC
AGTTTCAAAA GCTTCTGACT GAATTCAACA AATCCACTGA TGCATATGAG
CTGAAGATCG CCAACAAGCT CTTCGGAGAA AAAACGTATC TATTTTTACA
GGAATATTTA GATGCCATCA AGAAATTTTA CCAGACCAGT GTGGAATCTG
TTGATTTTGC AAATGCTCCA GAAGAAAGTC GAAAGAAGAT TAACTCCTGG
GTGGAAAGTC AAACGAATGA AAAAATTAAA AACCTAATTC CTGAAGGTAA
TATTGGCAGC AATACCACAT TGGTTCTTGT GAACGCAATC TATTTCAAAG
GGCAGTGGGA GAAGAAATTT AATAAAGAAG ATACTAAAGA GGAAAAATTT
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TACCATACAA AGGCAAAGAT CTAAGCATGA TTGTGTTGCT GCCAAATGAA
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GGAATGGACA AGTTTGCAGA ATATGAGAGA GACATGTGTC GATTTACACT
TACCTCGGTT CAAAATGGAA GAGAGCTATG ACCTCAAGGA CACGTTGAGA
ACCATGGGAA TGGTGAATAT CTTCAATGGG GATGCAGACC TCTCAGGCAT
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TGGAGGTCAC TGAGGAGGGA GTGGAAGCTG CAGCTGCCAC CGCTGTAGTA
GTAGTCGAAT TATCATCTCC TTCAACTAAT GAAGAGTTCT GTTGTAATCA
CCCTTTCCTA TTCTTCATAA GGCAAAATAA GACCAACAGC ATCCTCTTCT
ATGGCAGATT CTCATCCCCA

14. A DNA sequence according to claim 13, wherein the DNA sequence is CGCTGTAGTA GTAGTCGAAT TATCATCTCC TTCAACT.

15. Plasmid comprising the nucleotide sequence corresponding to one or more of exons 2-7 of SCCA1 gene fused to exon 8 of SCCA2 gene.

16. Plasmid comprising the nucleotide sequence corresponding to exons 2 -7 of SCCA1 fused to the nucleotide sequence of exon 8 of SCCA2.

17. Plasmid comprising the nucleotide sequence corresponding to one or more of exons 2-7 of SCCA2 gene fused to exon 8 of SCCA1 gene.

18. Plasmid comprising the nucleotide sequence corresponding to exons 2-7 of SCCA2 gene fused to exon 8 of SCCA1 gene.

19. Plasmid according to claims 15-16, comprising the nucleotide sequence of claim 13, and deposited at ECACC under deposition number ECACC 01031315.

20. Protein expression system for production of SCCA1/SCCA2 fusion protein

21. Recombinant bacteria comprising a plasmid according to claims 15-19.

22. Recombinant bacteria comprising a plasmid according to claim 16.

23. Recombinant E. coli comprising a plasmid according to claim 15.

24. Recombinant E. coli comprising a plasmid according to claim 16.

25. Method for detecting the gene rearrangement forming the SCCA1/SCCA2 fusion protein using a cDNA cloning and sequencing analysis of tumor DNA.

26. Method for detecting the gene rearrangement forming the SCCA2/SCCA1 fusion protein using a cDNA cloning and sequencing analysis of tumor DNA.

27. Method for detecting the gene rearrangement forming the SCCA1/SCCA2 fusion protein using a Southern blot-technology applied on tumor DNA.

28. Method for detecting the gene rearrangement forming the SCCA2/SCCA1 fusion protein using a Southern blot-technology applied on tumor DNA.

29. Method for detecting the gene rearrangement forming the SCCA1/SCCA2 fusion protein using a PCR-analysis technology.

30. Method for detecting the gene rearrangement forming the SCCA2/SCCA1 fusion protein using a PCR-analysis technology.

31. Method for detecting the gene rearrangement forming the SCCA1/SCCA2 fusion protein using an amino acid sequencing technology.

32. Method for detecting the gene rearrangement forming the SCCA2/SCCA1 fusion protein using an amino acid sequencing technology.

33. Method for detection the SCCA1/A2 fusion protein using Western blotting

34. Method for detection the SCCA2/A1 fusion protein using Western blotting

35. Monoclonal antibody specific for SCCA1/SCCA2 fusion protein.

36. Monoclonal antibody specific for SCCA2/SCCA1 fusion protein.

37. Polyclonal antibody reactive with SCCA1/SCCA2 fusion protein.

38. Monoclonal antibody specific for SCCA2/SCCA1 fusion protein.

39. Immunoassay using a monoclonal antibody or polyclonal antibody specific for SCCA1/SCCA2 fusion protein for detecting the presence and concentration of SCCA1/SCCA2 fusion protein.

40. Immunoassay using a monoclonal antibody or polyclonal antibody specific for SCCA2/SCCA1 fusion protein for detecting the presence and concentration of SCCA2/SCCA1 fusion protein.

41. Method for diagnosing the presence or absence of a squamous cell carcinoma by detecting the SCCA1/SCCA2 fusion protein in a human sample.

42. Method for diagnosing the presence or absence of a squamous cell carcinoma by detecting the SCCA2/SCCA1 fusion protein in a human sample.

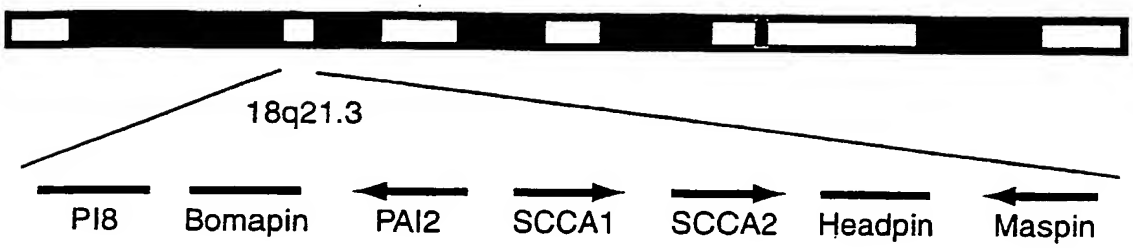
43. Method according to claims 41-42, wherein the fusion protein is used in a histochemical analysis.

44. Kit comprising a SCCA1/SCCA2 fusion protein antibody to be used in the determination of the presence or absence of squamous cell carcinoma (SCC).

45. Kit comprising a SCCA2/SCCA1 fusion protein antibody to be used in the determination of the presence or absence of squamous cell carcinoma (SCC).

46. Kit according to claim 44-45, in that it further comprises antibodies related to SCCA1 and/or SCCA2.

A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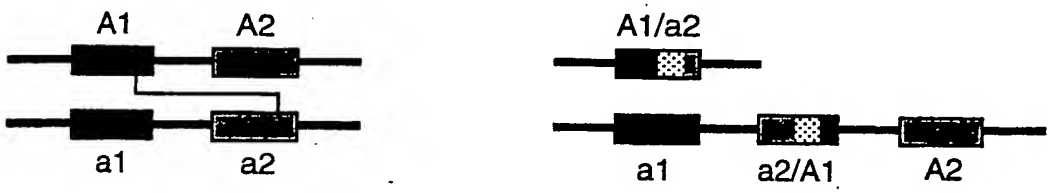
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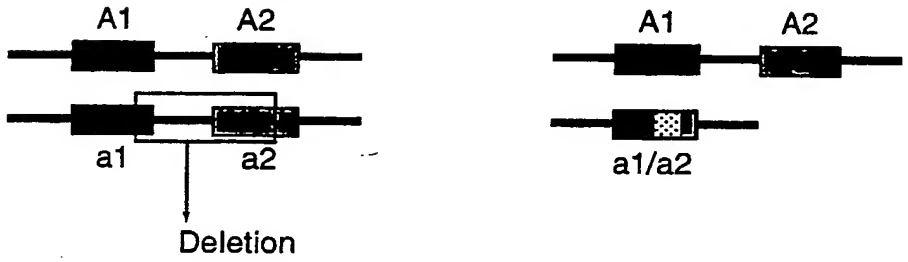
A2
a1
a2
A1/a2
a1
a2/A1
A2

C


A1
A2
a1
a2
Deletion
A1
A2
a1/a2

SCCA1 I1-ATGAATTCAC TCAGTGAAGC CAACACCAAG TTCATGTTCG ACCTGTTCCA ACAGTTCAGA
SCCA2 I1-ATGAATTCAC TCAGTGAAGC CAACACCAAG TTCATGTTCG ATCTGTTCCA ACAGTTCAGA

AAATCAAAAG AGAACAACAT CTTCTATTCC CCTATCAGCA TCACATCAGC ATTAGGGATG
AAATCAAAAG AGAACAACAT CTTCTATTCC CCTATCAGCA TCACATCAGC ATTAGGGATG

GTCCTCTTAG GAGCCAAAGA CAACACTGCA CAACAGATTA AGAAG-I2-GTTCT TCACTTTGAT
GTCCTCTTAG GAGCCAAAGA CAACACTGCA CAACAAATTA GCAAG-I2-GTTCT TCACTTTGAT

CAAGTCACAG AGAACACCAC AGGAAAAGCT GCAACATATC AT-I3-GTTGATAG GTCAGGAAAT
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CTGAAGATCG CCAACAAGCT CTTCGGAGAA AAAACGTATC TATTTTTACA G-I4-GAATATTTA
CTGAAGATCG CCAACAAGCT CTTCGGAGAA AAGACGTATC AATTTTTACA G-I4-GAATATTTA

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ATCCTCTTCT ATGGCAGATT CTCATCCCCG TAG
ATCCTCTTCT ATGGCAGATT CTCATCCCCA TAG

SCCA1 MNSLSEANTK FMFDLFQQFR KSKENNIFYS PISITSALGM VLLGAKDNTA
SCCA2 MNSLSEANTK FMFDLFQQFR KSKENNIFYS PISITSALGM VLLGAKDNTA

QQI**K**KVLHFD QVTENTT**G**KA ATYHVDRSGN VHHQFQKLLT EFNKSTDAYE
QQI**S**KVLHFD QVTENTT**E**KA ATYHVDRSGN VHHQFQKLLT EFNKSTDAYE

LKIANKLFGE KTY**L**FLQEYL DAIKKFYQTS VES**V**DFANAP EESRKKINSW
LKIANKLFGE KTY**Q**FLQEYL DAIKKFYQTS VES**T**DFANAP EESRKKINSW

VESQTNEKIK NL**I**P**E**G**N**IG**S** **N**TTLVLVNAI YFKGQWE**K**KF **N**KE**D**TKEEKF
VESQTNEKIK NL**F**P**D**G**T**IG**N** **D**TTLVLVNAI YFKGQWE**N**KF **K**KE**N**TKEEKF

WPNKNTYKS**I** QMMRQY**T**SF**H** FA**S**LEDVQAK VLEIPYKGKD LSMIVLLPNE
WPNKNTYKS**V** QMMRQY**N**SF**N** FA**L**LEDVQAK VLEIPYKGKD LSMIVLLPNE

IDGLQKLEEK LTAEKLMEWT SLQNMRET**R**V DLHLPRFK**V**E ESYDLKDTLR
IDGLQKLEEK LTAEKLMEWT SLQNMRET**C**V DLHLPRFK**M**E ESYDLKDTLR

TMGMV**D**IFNG DADLSGMT**G**S **R**GL**VL**S**G**VLH KAFVEVT*EEG* ***A****EAAAAA*<u>*TAVV*</u>
TMGMV**N**IFNG DADLSGMT**W**S **H**GL**SV**S**K**VLH KAFVEVT*EEG* ***V****EAAAAA*<u>*TAVV*</u>

<u>***GFGSSPAST***</u>N EEF**H**CNHPFL FFIRQNKTNS ILFYGRFSSP
<u>***VVELSSPST***</u>N EEF**C**CNHPFL FFIRQNKTNS ILFYGRFSSP

SCCA1A2 cDNA sequence

ATGAATTCAC TCAGTGAAGC CAACACCAAG TTCATGTTCG ACCTGTTCCA ACAGTTCAGA

AAATCAAAAG AGAACAACAT CTTCTATTCC CCTATCAGCA TCACATCAGC ATTAGGGATG

GTCCTCTTAG GAGCCAAAGA CAACACTGCA CAACAGATTA AGAAGGTTCT TCACTTTGAT

CAAGTCACAG AGAACACCAC AGGAAAAGCT GCAACATATC ATGTTGATAG GTCAGGAAAT

GTTCATCACC AGTTTCAAAA GCTTCTGACT GAATTCAACA AATCCACTGA TGCATATGAG

CTGAAGATCG CCAACAAGCT CTTCGGAGAA AAAACGTATC TATTTTTACA GGAATATTTA

GATGCCATCA AGAAATTTTA CCAGACCAGT GTGGAATCTG TTGATTTTGC AAATGCTCCA

GAAGAAAGTC GAAAGAAGAT TAACTCCTGG GTGGAAAGTC AAACGAATGA AAAAATTAAA

AACCTAATTC CTGAAGGTAA TATTGGCAGC AATACCACAT TGGTTCTTGT GAACGCAATC

TATTTCAAAG GGCAGTGGGA GAAGAAATTT AATAAAGAAG ATACTAAAGA GGAAAAATTT

TGGCCAAACA AGAATACATA CAARTCYRTA CAGATGATGA GGCAATACAM WTCYTTTMAT

TTTGCCTYGC TGGAGGATGT ACAGGCCAAG GTCCTGGAAA TACCATACAA AGGCAAAGAT

CTAAGCATGA TTGTGYTGCT GCCAAATGAA ATCGATGGTC TSCAGAAGCT TGAAGAGAAA

CTCACTGCTG AGAAATTGAT GGAATGGACA AGTTTGCAGA ATATGAGAGA GACAYGTGTC

GATTTACACT TACCTCGGTT CAAARTGGAA GAGAGCTATG ACCTCAAGGA CACGTTGAGA

ACCATGGGAA TGGTGRATAT CTTCAATGGG GATGCAGACC TCTCAGGCAT GACCKGGAGC

CRCGGTCTCK YRSTATCTRR AGTCCTACAC AAGGCCTTTG TGGAGGTYAC WGAGGAGGGA

GYRGAAGCTG CAGCTGCCAC CGCTGTAGTA GTAGTCGAAT TATCATCTCC TTCAACTAAT

GAAGAGTTCT GTTGTAATCA CCCTTTCCTA TTCTTCATAA GGCAAAATAA GACCAACAGC

ATCCTCTTCT ATGGCAGATT CTCATCCCCA TAG

SCCA1A2 amino acid sequence

MNSLSEANTK FMFDLFQQFR KSKENNIFYS PISITSALGM VLLGAKDNTA

QQIKKVLHFD QVTENTTGKA ATYHVDRSGN VHHQFQKLLT EFNKSTDAYE

LKIANKLFGE KTYLFLQEYL DAIKKFYQTS VESVDFANAP EESRKKINSW

VESQTNEKIK NLIPEGNIGS NTTLVLVNAI YFKGQWEKKF NKEDTKEEKF

WPNKNTYKS$_{I/V}$ QMMRQY$_{T/N}$SF$_{H/N}$ FA$_{S/L}$LEDVQAK VLEIPYKGKD LSMIVLLPNE

IDGLQKLEEK LTAEKLMEWT SLQNMRET$_{R/C}$V DLHLPRFK$_{V/M}$E ESYDLKDTLR

TMGMVD/NIFNG DADLSGMTG/WS R/HGLV/SL/VSG/KVLH KAFVEVTEEG
A/VEAAAATAVV

VVELSSPSTN EEFCCNHPFL FFIRQNKTNS ILFYGRFSSP

SCCA2A1 cDNA sequence

ATGAATTCAC TCAGTGAAGC CAACACCAAG TTCATGTTCG ATCTGTTCCA ACAGTTCAGA
AAATCAAAAG AGAACAACAT CTTCTATTCC CCTATCAGCA TCACATCAGC ATTAGGGATG
GTCCTCTTAG GAGCCAAAGA CAACACTGCA CAACAAATTA GCAAGGTTCT TCACTTTGAT
CAAGTCACAG AGAACACCAC AGAAAAAGCT GCAACATATC ATGTTGATAG GTCAGGAAAT
GTTCATCACC AGTTTCAAAA GCTTCTGACT GAATTCAACA AATCCACTGA TGCATATGAG
CTGAAGATCG CCAACAAGCT CTTCGGAGAA AAGACGTATC AATTTTTACA GGAATATTTA
GATGCCATCA AGAAATTTTA CCAGACCAGT GTGGAATCTA CTGATTTTGC AAATGCTCCA
GAAGAAAGTC GAAAGAAGAT TAACTCCTGG GTGGAAAGTC AAACGAATGA AAAAATTAAA
AACCTATTTC CTGATGGGAC TATTGGCAAT GATACGACAC TGGTTCTTGT GAACGCAATC
TATTTCAAAG GGCAGTGGGA GAATAAATTT AAAAAAGAAA ACACTAAAGA GGAAAAATTT
TGGCCAAACA AGAATACATA CAARTCYRTA CAGATGATGA GGCAATACAM WTCYTTTMAT
TTTGCCTYGC TGGAGGATGT ACAGGCCAAG GTCCTGGAAA TACCATACAA AGGCAAAGAT
CTAAGCATGA TTGTGYTGCT GCCAAATGAA ATCGATGGTC TSCAGAAGCT TGAAGAGAAA
CTCACTGCTG AGAAATTGAT GGAATGGACA AGTTTGCAGA ATATGAGAGA GACAYGTGTC
GATTTACACT TACCTCGGTT CAAARTGGAA GAGAGCTATG ACCTCAAGGA CACGTTGAGA
ACCATGGGAA TGGTGRATAT CTTCAATGGG GATGCAGACC TCTCAGGCAT GACCKGGAGC
CRCGGTCTCK YRSTATCTRR AGTCCTACAC AAGGCCTTTG TGGAGGTYAC WGAGGAGGGA
GYRGAAGCTG CAGCTGCCAC CGCTGTAGTA GGATTCGGAT CATCACCTAC TTCAACTAAT
GAAGAGTTCC ATTGTAATCA CCCTTTCCTA TTCTTCATAA GGCAAAATAA GACCAACAGC
ATCCTCTTCT ATGGCAGATT CTCATCCCCG TAG

SCCA2A1 amino acid sequence

MNSLSEANTK FMFDLFQQFR KSKENNIFYS PISITSALGM VLLGAKDNTA

QQISKVLHFD QVTENTTEKA ATYHVDRSGN VHHQFQKLLT EFNKSTDAYE

LKIANKLFGE KTYQFLQEYL DAIKKFYQTS VESTDFANAP EESRKKINSW

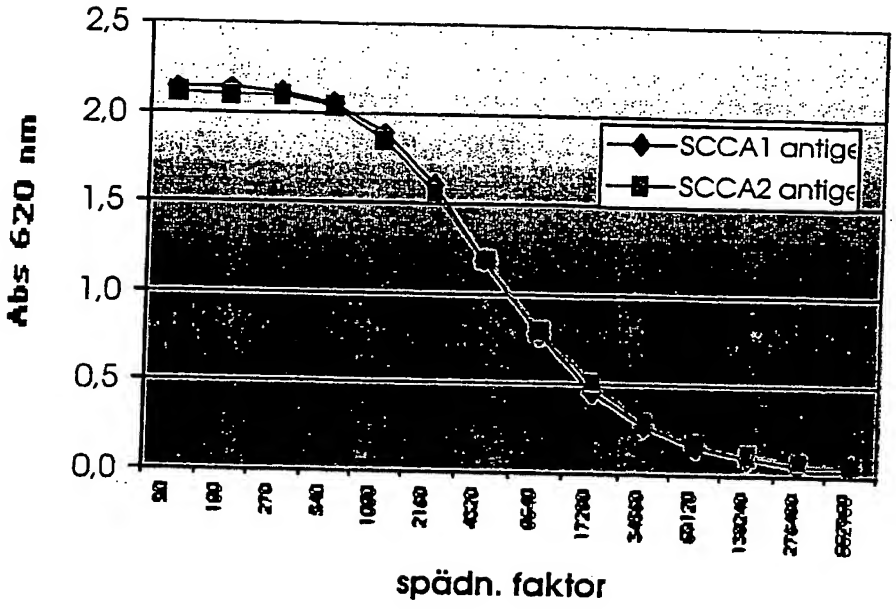
VESQTNEKIK NLFPDGTIGN DTTLVLVNAI YFKGQWENKF KKENTKEEKF

WPNKNTYKS$_{I/V}$ QMMRQY$_{T/N}$SF$_{H/N}$ FA$_{S/L}$LEDVQAK VLEIPYKGKD LSMIVLLPNE

IDGLQKLEEK LTAEKLMEWT SLQNMRET$_{R/C}$V DLHLPRFK$_{V/M}$E ESYDLKDTLR

TMGMVD/NIFNG DADLSGMTG/WS R/HGLV/SL/VSG/KVLH KAFVEVTEEG
A/VEAAAATAVV

GFGSSPASTN EEFHCNHPFL FFIRQNKTNS ILFYGRFSSP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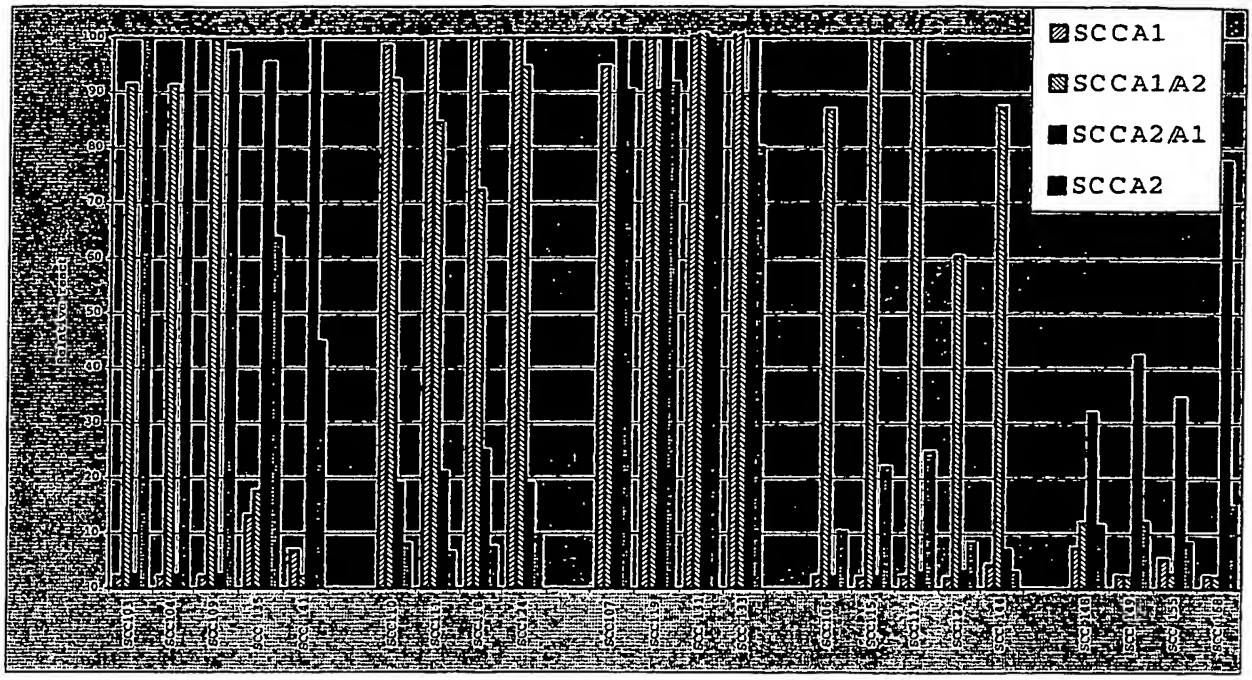
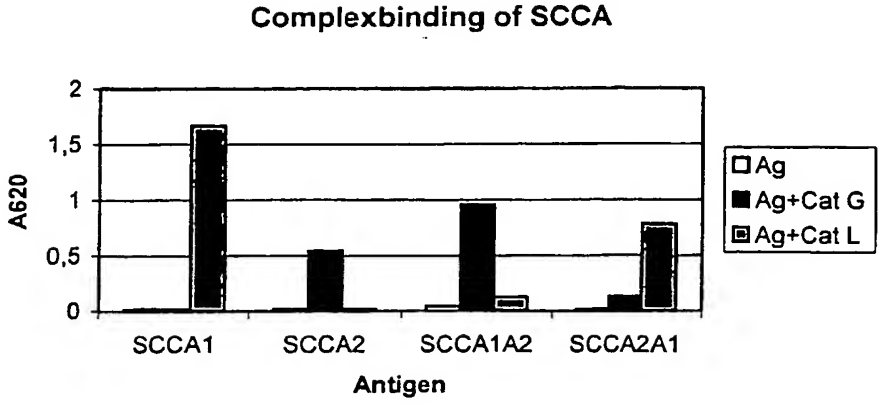
1,5
1,0
0,5
0,0
Abs 620 nm
SCCA1 antige
SCCA2 antige
spädn. faktor


SCCA1
SCCA1/A2
SCCA2/A1
SCCA2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1,5
1
0,5
0
A620
SCCA1
SCCA2
SCCA1A2
SCCA2A1
Antigen
Ag
Ag+Cat G
Ag+Cat L

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(74) Agent: **STRÖM & GULLIKSSON IP AB**; Sjörporten 4, S-417 64 Göteborg (SE).

(81) Designated States *(national)*: AE, AG, AL, AM, AT (utility model), AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ (utility model), CZ, DE (utility model), DE, DK (utility model), DK, DM, DZ, EC, EE (utility model), EE, ES, FI (utility model), FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK (utility model), SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
14 November 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: REARRANGED SQUAMOUS CELL CARCINOMA ANTIGEN GENES II

SCCA1A2 amino acid sequence

MNSLSEANTK FMFDLFQQFR KSKENNIFYS PISITSALGM VLLGAKDNTA

QQIKKVLHFD QVTENTTGKA ATYHVDRSGN VHHQFQKLLT EFNKSTDAYE

LKIANKLFGE KTYLFLQEYL DAIKKFYQTS VESVDFANAP EESRKKINSW

VESQTNEKIK NLIPEGNIGS NTTLVLVNAI YFKGQWEKKF NKEDTKEEKF

WPNKNTYKS$_{I/V}$ QMMRQY$_{T/N}$SF$_{H/N}$ FA$_{S/L}$LEDVQAK VLEIPYKGKD LSMIVLLPNE

IDGLQKLEEK LTAEKLMEWT SLQNMRET$_{R/C}$V DLHLPRFK$_{V/M}$E ESYDLKDTLR

TMGMVD/NIFNG DADLSGMTG/WS R/HGLV/SL/VSG/KVLH KAFVEVTEEG
A/VEAAAATAVV

VVELSSPSTN EEFCCNHPFL FFIRQNKTNS ILFYGRFSSP

(57) Abstract: The present invention relates to a SCCA1/SCCA2 fusion protein; plasmid containing the same; antibodies of said fusion protein; methods for detecting said protein; methods for diagnosing th epresence or absence of SCC by determining the presence of SCCA1/SCCA2 fusion protein.

WO 02/074904 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 02/00512

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12N 15/15
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, WPI DATA, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCHICK, Charles et al. "The reactive site loop of the serpin SCCA1 is essential for cysteine proteinase inhibition". Proc. Natl. Acad. Sci. USA, November 1998, Vol. 95, pages 13465-13470, table 1.	10,14
A	table 1.	6,8-9,12-13, 16,18-19
	--	
X	SCHICK, Charles et al. "Squamous Cell Carcinoma Antigen 2 Is a Novel Serpin That Inhibits the Chymotrypsin-like Proteinases Cathepsin G and Mast Cell Chymase". The Journal of Biological Chemistry, January 17 1997, Vol. 272, No. 3, pp. 1849-1855, figure 1.	10,14
A		6,8-9,12-13, 16,18-19
	--	

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 July 2002	12 -07- 2002
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Frida Plym Forshell /OGU Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 02/00512

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE 19742725 A1 (ABTS, HARRY FRANK, DR.), 1 April 1999 (01.04.99)	6,8-10, 12-14,16, 18-19
&	Derwent GeneSeq database of patented sequences, Acc. No. AAY25928	
&	Derwent GeneSeq database of patented sequences, Acc. No. AAY25927	
	--	
A	WO 0102603 A1 (STENMAN, JAKOB), 11 January 2001 (11.01.01), abstract	6,8-10, 12-14,16, 18-19
	--	
P,A	SUMINAMI, Yoshinori et al. "Novel forms of squamous cell carcinoma antigen transcripts produced by alternative splicing". Biochimica et Biophysica Acta 1519, 2001, pp. 122-126, abstract	6,8-10, 12-14,16, 18-19
	--	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 02/00512

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 1-5,7,11,15,17,20-46
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

see extra sheet

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

International application No.
PCT/SE 02/00512

Present claims 1-5, 7, 11, 15, 17 and 20-46 relate to an extremely large number of possible sequences, methods and kits. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the sequences, methods and kits claimed. In the present case, the claims so lack support that a meaningful search over the whole claimed scope is impossible.

Consequently, a search has been carried out for the parts of the claims which appear to be supported and disclosed, namely the parts specified in dependent claims 6, 8-10 and 12-14, 16 and 18-19. In these claims it is specified which parts on which genes (exon 2-7 of SCCA1/SCCA2 and exon 8 of SCCA2/SCCA1) that are fused, which is a prerequisite for a meaningful search.

INTERNATIONAL SEARCH REPORT

Information on patent family members

10/06/02

International application No.
PCT/SE 02/00512

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 19742725 A1	01/04/99	NONE	
WO 0102603 A1	11/01/01	AU 5830400 A	22/01/01